Detection, Confirmation, and Quantification of Chloramphenicol in Honey and Shrimp at Regulatory Levels Using Quadrupole and Ion Trap LC/MS

Application

Foods, Environmental

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Abstract

Methodology capable of meeting regulatory requirements has been developed for the determination of chloramphenicol in honey and shrimp. Samples of the two foodstuffs are extracted with Isolute HN-M cartridges and analyzed with both the Agilent 1100 LC/MSD Trap (SL) and the Agilent 1100 LC/MSD (SL) quadrupole with negative mode electrospray ionization. Using deuterated internal standard and one simple sample extraction procedure, both instruments provide a limit of detection at or below 0.1 ppb in both shrimp and honey. Detection limits are lower using the ion trap for shrimp because of less matrix interference. The Agilent 1100 LC/MSD gives quantitative results and the Agilent 1100 LC/MSD Trap gives full spectrum confirmation.

Introduction

Chloramphenicol is a broad range antibiotic that has found its way into foodstuffs such as honey and shrimp. Because it has displayed significant toxicological effects on humans, it has been banned from foods in the European community and the United States at levels greater than 0.1 ppb. Analytical methods used to determine this limit must achieve both the required sensitivity and maintain sufficient selectively. LC/MS has been demonstrated by the US Food and Drug Administration for these analysis [1-3]. In addition, the Commission of European Communities has issued guidelines stipulating that for mass spectral detection, a molecular ion (or quasimolecular ion) and at least two fragment ions are needed for positive confirmation [4]. For quantitative analysis the Agilent 1100 LC/MSD provides excellent results and can give some confirmation information. The Agilent 1100 LC/MSD Trap gives excellent full spectrum confirmation at the regulated concentration.

Experimental

Reagents and Materials

ISOLUTE HM-N cartridges from IST (Hengoed, UK, Part-nr. 800-1300-FM)

Ethyl acetate from Vel (Merck Eurolab, Leuven, Belgium)

Methanol HPLC-grade from Merck (LiChrosolv, Darmstadt, Germany)

Deuterated (d5) CAP internal standard from Cambridge Isotope Laboratories (CIL, Andover, MA, USA)

Syringe filters (0.2 µm, PTFE) from Alltech Associates Inc. (Lokeren, Belgium)

Sample Preparation

For honey, 5 g of sample is diluted to 20 mL with water and 5 µL of 1 ng/µL internal standard (IS) is added. The solution is loaded on the cartridge and allowed to stand for 5 minutes. Elution is performed with 50 mL ethyl acetate. The eluate is collected and the solvent is evaporated under a nitrogen stream at 40 °C. The residue is redissolved in 1 mL water/methanol (9/1, v/v) and put in an ultrasonic bath for 1 minute. The solution is filtered, using a syringe filter, before injection. No additional clean-up of the sample solution is performed.

For shrimp, a portion of at least 10 g of frozen shrimp is defrosted and mixed in a blender. To 10 g of the mixed shrimp, 30 mL of water and 10 µL of 1 ng/µL IS is added. This portion is centrifuged for 10 minutes (2000 rpm). A 20-mL portion of the supernatant is loaded on the cartridge and allowed to stand for 5 minutes. Elution is performed with 50 mL ethyl acetate. The eluate is collected and the solvent evaporated under a nitrogen stream at 40 °C. The residue is redissolved in 1 mL water/methanol (9/1, v/v) and put in an ultrasonic bath for 1 minute. The solution is filtered before injection.

LC/MS Conditions

The LC/MS systems were the Agilent 1100 LC/MSD quadrupole mass spectrometer and the Agilent 1100 LC/MSD Trap. Both were equipped with Agilent 1100 binary pumps and 1100 well plate autosamplers. See Table 1.

Table 1. LC/MS Conditions

HPLC		
Column	Eclipse XDB C18, 4.6 mm × 150 mm, 5 µm (p/n 993967.902)	
Flow-rate	0.9 mL/min	
Mobile phase	10 mM ammonium acetate in water (solvent A) Methanol/acetonitrile 1/9 (solvent B) both from Merck (LiChrosolv, Darmstadt, Germany)	
Gradient	0–1 min	30% B
	1–8 min	30%–70% B
	8–8.5 min	70%–100% B
	8.5–12 min	100% B
	Post time	4 min at 30% B
Injection	100 µL with needle wash (methanol)	
Injection solvent	Water/methanol (9/1 v/v) for both standards and samples	
Column temperature	30 °C	
MSD source settings		
Source	ESI	
Ion polarity	Negative	
Drying gas temperature	340 °C	
Drying gas flow-rate	11 L/min	
Nebulizer pressure	50 psig	
V_{cap}	3500 V	
Quadrupole MSD		
MSD acquisition on	Between 3 and 7.5 min	
Fragmentor	160 V	
SIM settings	*m/z* 257, 321, 323 (CAP) *m/z* 262, 326, 328 (CAP-d5)	

Table 1. LC/MS Conditions (continued)

Trap MSD	
MSD acquisition on	Between 3 and 7.5 min
Target mass (SPS)	323 *m/z*
Trap parameters	
Max. accumulation time	300 ms
ICC target	30,000
Scan range	160–340
Averaging	2
Fragmentation parameters (MS/MS)	
Smart Frag	On, 30%–200% (default)
Isolation mass	*m/z* 325.0
Isolation width	10.0 *m/z*
Fragmentation amplitude	1.0 V
Fragmentation cutoff	*m/z* 88

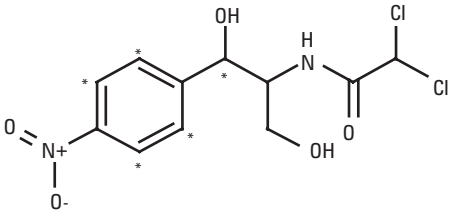
Results and Discussion

Spectral Quality and Sensitivity of Standards

For analysis with the quadrupole LC/MSD, selected ion monitoring (SIM) was used to obtain the required sensitivity. Table 2 shows the structure, fragment ions and identity of CAP and CAP-d5. Figure 1 shows the analysis of a standard mixture containing 2.5 pg/µL CAP and 5 pg/µL CAP-d5. By applying a fragmentor voltage of 160 V, fragment ions at *m/z* 257 and 262 are detected for confirmation purposes. Lowering the fragmentor voltage to optimize for the *m/z* 321 and *m/z* 326 and monitoring those ion alone would obtain greater sensitivity. However, the confirmation of the fragment ions would be lost. For screening analysis without confirmation this would be acceptable and provide a much lower limit of detection (LOD).

Table 2 Structure and Fragment Ions and Identity of CAP and CAP-d5 (* Indicates Deuterated Positions for the CAP-d5 IS)

Chloramphenicol structure



	m/z	Identity
CAP	257	$[M-H-HCOCl]^-$
	249	$[M-H-2HCl]^-$
	194	$[M-H-NH2CoCl2H]^-$
	176	$[M-H-NH2CoCl2H-H2O]^-$
CAP-d5	262	$[M-H-HCOCl]^-$
	254	$[M-H2HCl]^-$
	199	$[M-H-NH_2COCl_2H]^-$
	180	$[M-H-NH_2COCl_2H-HDO]^-$

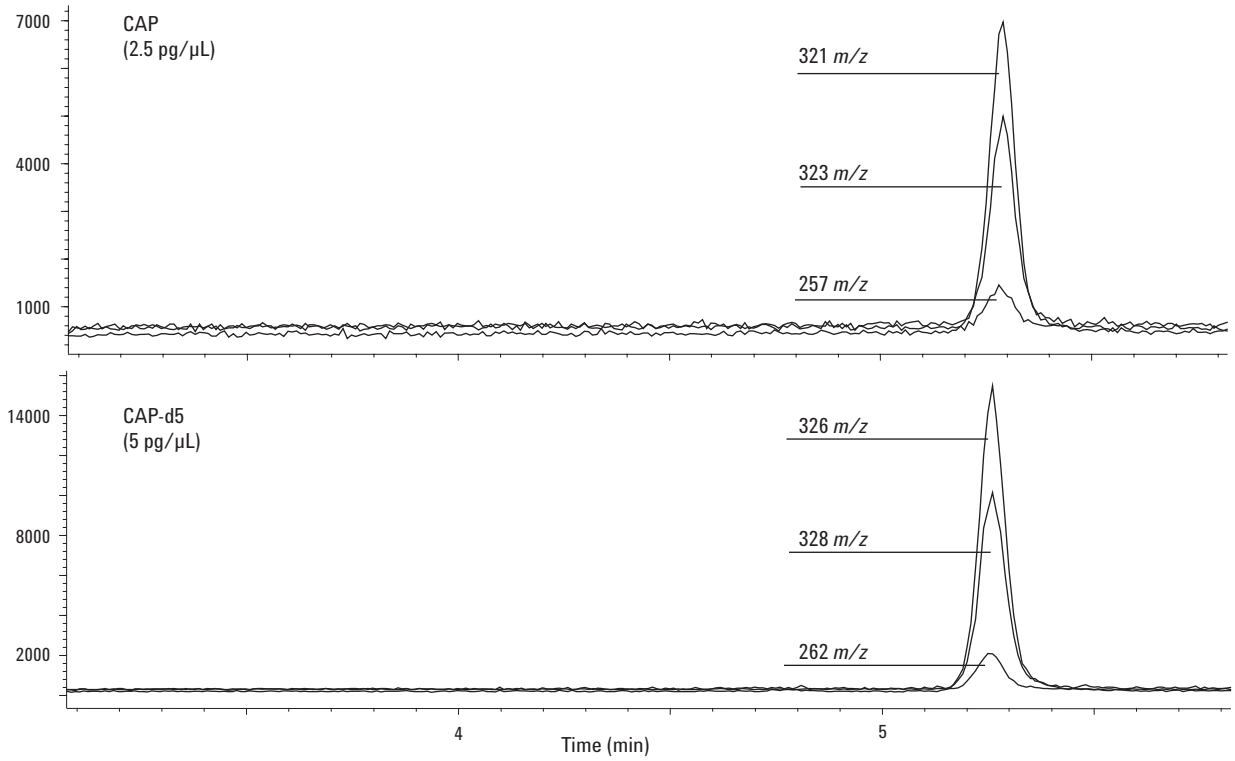


Figure 1. Analysis of a standard solution containing 2.5 ppb of CAP and 5 ppb of CAP-d5 (IS) on the quadrupole MSD. The extracted ion chromatogram for the corresponding ions are shown.

Using the LC/MSD Trap in MS/MS mode both the needed sensitivity (through reduction in chemical noise) and selectivity (for confirmation) is obtained. The compound shows a clear and reproducible fragmentation pattern. An example of the analysis of the standard mixture together with the corresponding MS/MS spectra is shown in Figure 2. Optimizing the fragmentation energy [turning off Smart Frag] and fragmentation cutoff in the ion trap will increase sensitivity even further than shown here. Using an isolation width of 10 *m/z* allows inclusion of the chlorine isotopes in the resulting full scan mass spectra of the analyte and the Cl^{35} isotope of the internal standard. Contact Agilent for more details on these and other ion trap settings.

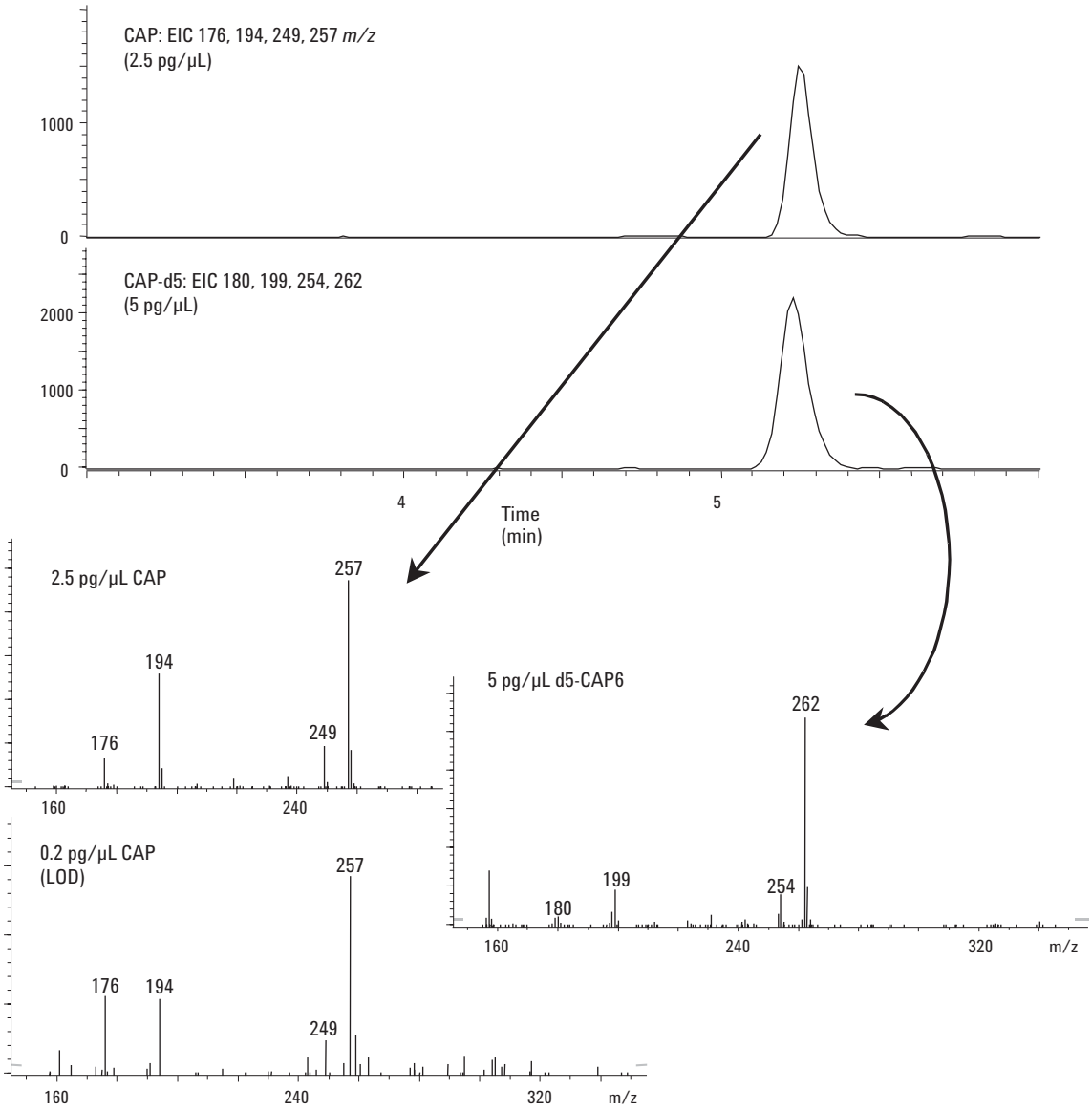


Figure 2. Analysis of a standard solution containing 2.5 pg/µL CAP and 5 pg/µL CAP-d5 (IS) on the LC/MSD Trap together with the corresponding MS/MS spectra and the MS/MS spectrum resulting from an analysis of a standard solution containing 0.2 pg/µL CAP.

Method Performance

Standard solutions of CAP containing 5 pg/µL of CAP-d5 were injected six consecutive times to test repeatability of injection on the mass selective detector (MSD) quadrupole instrument. This was done at two concentration levels. Each time, the response of CAP relative to CAP-d5 was recorded. For a solution containing 0.5 pg/µL CAP the relative standard deviations (RSDs) on the relative response were 5.05%. This 0.5-pg/µL level would correspond to a sample containing approximately 0.1 ppb CAP with the five-fold concentration step. When a solution containing 5 pg/µL CAP was analyzed, RSDs on the relative response were 1.28% for the quadrupole.

A calibration line was constructed by injecting standard solutions of CAP with a concentration of 0 to 25 pg/µL with 5 pg/µL of the IS added to each solution. One injection was performed per concentration. The quadrupole showed a linear response for CAP in this concentration range. Calibration curves and correlation coefficients are shown in Figure 3. The LOD with this method was determined to be ca. 0.2 pg/µL in a standard solution for both mass spectrometers. With the 100-µL injection used, this corresponds with 20 pg on-column.

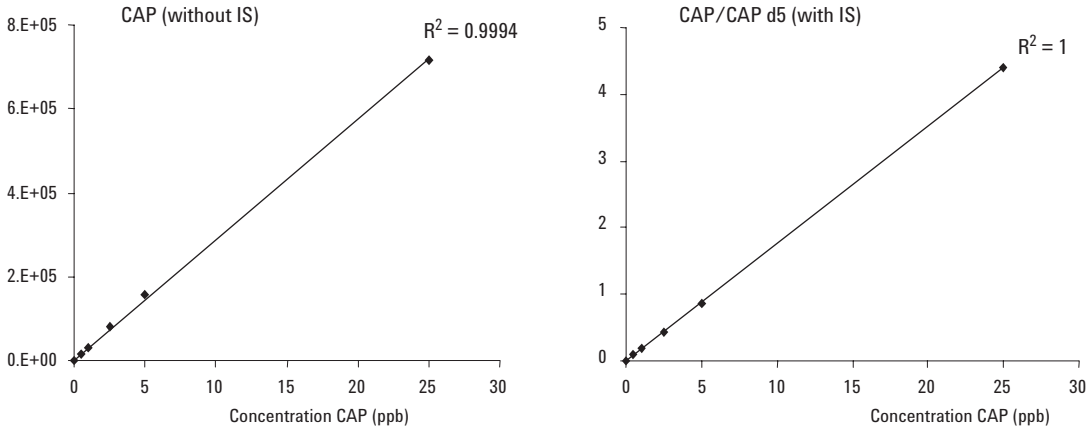


Figure 3. Calibration graphs for standard solutions of CAP on the quadrupole with and without CAP-d5 (IS).

Extraction Recovery and Repeatability of Extraction

The extraction procedure was evaluated on repeatability and linearity with the quadrupole instrument. Blank honey was spiked with 1 ppb CAP and 1 ppb CAP-d5. The extraction procedure was carried out six times and the recovery was calculated. The recovery for CAP varied from 85.31% to 94.94% and the mean recovery was 90.60%. The RSD on the recovery was 4.34% for CAP and 3.39% when the IS was taken into account. An analysis of blank honey spiked only with the IS is shown in Figure 4 run on both instruments. With the quadrupole, LC/MSD matrix interferences are present but chromatographically separated from the CAP signal. The ion trap results show that no matrix interference is present in the isolation window from *m/z* 318 to 328. The data suggest that other endogenous compounds in honey produce fragments at the same *m/z* as CAP. This supports an even lower detection limit for this matrix if a screening analysis were conducted with a lower fragmentor voltage monitoring only the *m/z* 321.

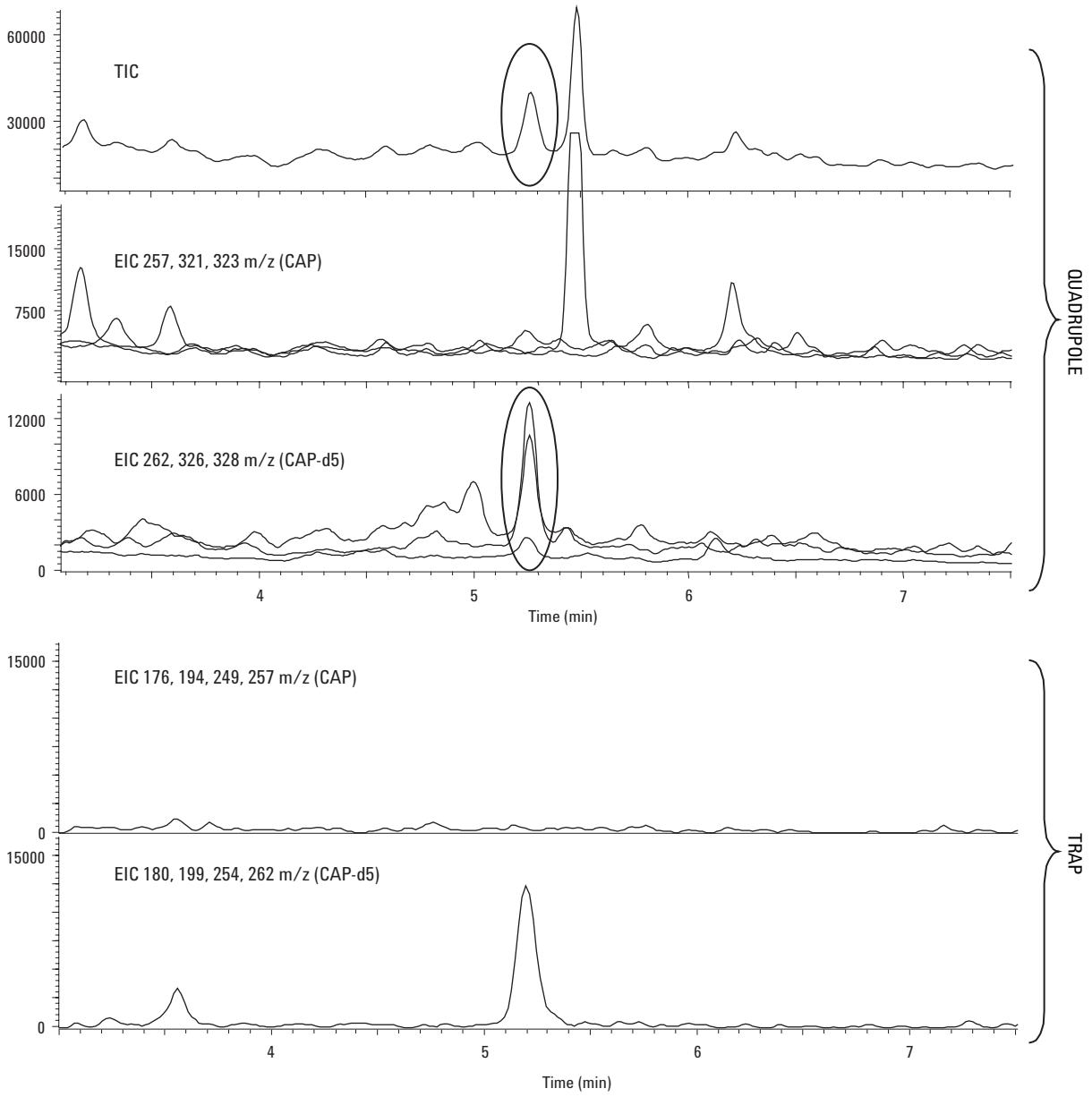


Figure 4. Analysis of a blank honey sample containing 1 ppb CAP-d5.

A calibration curve was constructed with blank honey samples spiked with 0, 0.1, 0.2, 0.5, 1.0, and 2.0 ppb CAP. The samples also contained 1 ppb of the IS. The correlation coefficients were 0.9997 and 0.9998 without and with correction with the IS, respectively. The slope for the calibration curve constructed with these extracts for CAP with correction with the IS was 0.1822. This is in good agreement with the slope obtained with the standard solutions, which is 0.1758 (see Figure 3).

Spectra on the trap were similar for standard solutions and real samples. An example of an MS/MS spectrum of an extract of a honey sample spiked with 0.5 ppb CAP and 1 ppb CAP-d5 is shown in Figure 5. Since the analyte and the IS coelute, a mixed spectrum is obtained. This could be avoided by using a smaller isolation width and the multiple reaction monitoring (MRM) function of the ion trap. Note that the chlorine isotope for $Cl^{35}Cl^{37}$ is not observed for the deuterated internal standard because its precursor ion is at the edge of the isolation width and thus not trapped.

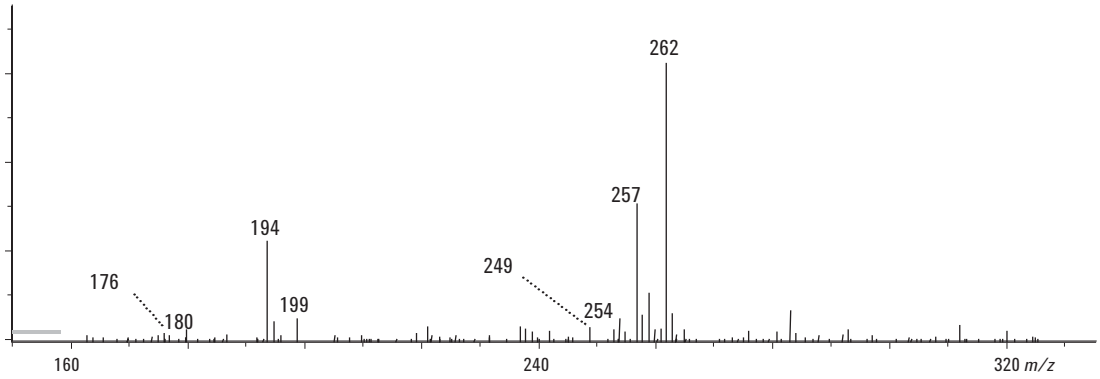


Figure 5. Ion trap MS/MS spectrum from analysis of a honey sample spiked with 0.5 ppb CAP and 1 ppb CAP-d5.

Analysis of Honey

The extraction procedure and LC/MS methods were applied to the analysis of honey samples that were known to contain CAP. Sample results obtained with the quadrupole and trap MSD were compared (Figure 6).

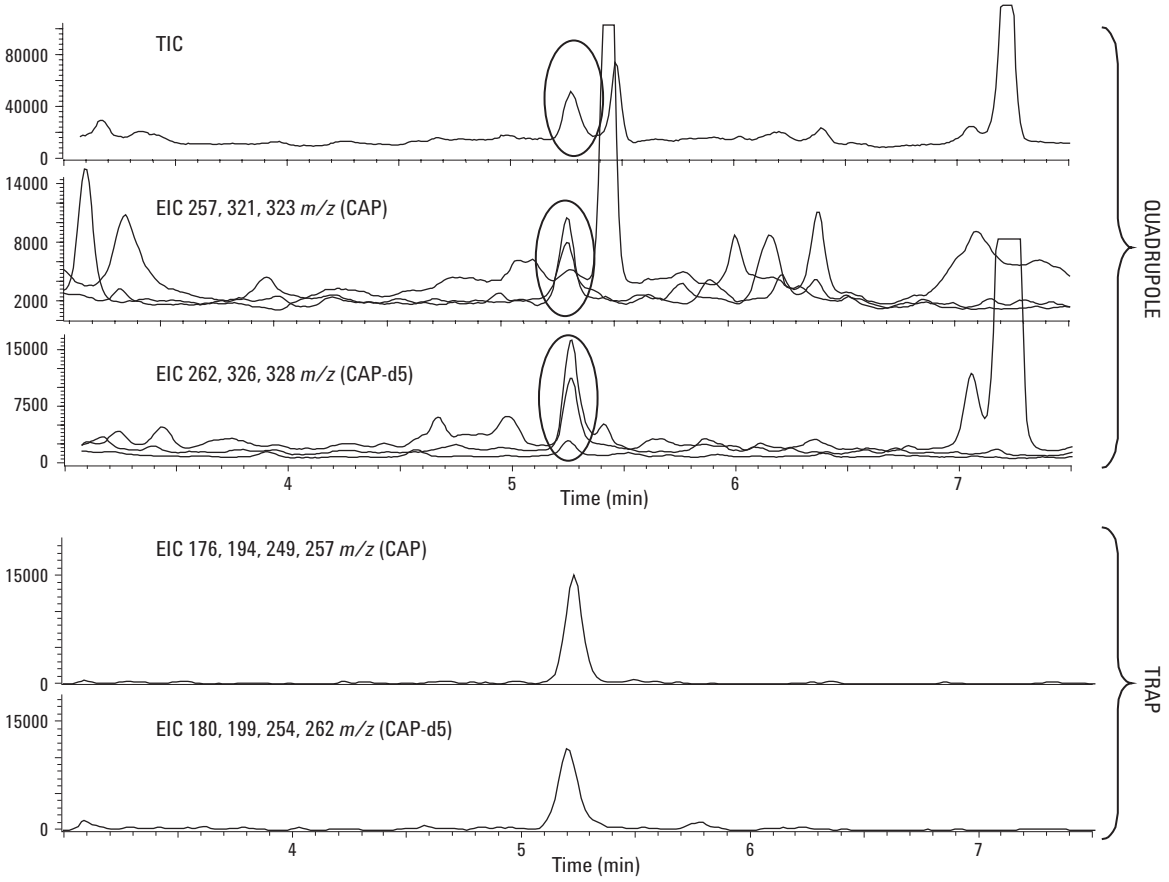


Figure 6. Analysis of a honey sample containing 0.5 ppb CAP and 1 ppb CAP-d5.

The LOD for the honey samples varies between detectors. For the quadrupole, it is found to be 0.5 pg/µL in the analytical solution. This corresponds with 50 pg on-column. Taking into account the sample preparation with a five-fold concentration, samples containing 0.1 ppb CAP can be detected. It is obvious that the sample matrix interferes with the sensitivity (Figures 4 and 6). Due to the increased selectivity using MS/MS in the trap, the LOD with this MS is similar for honey samples as for the standard solutions and is ca. 0.2 pg/µL in the analytical solution. This is equivalent to 0.04 ppb CAP in the sample because of the five-fold concentration step.

Analysis of Shrimp

The same sample preparation method was applied to the analysis of shrimp. The total volume of shrimp and water added was about 40 mL. Taking 20 mL of the 10 g shrimp aliquot for the Isolute sample preparation and reconstituting the dried extract in 1 mL produced a five-fold concentration as with the honey. This sample preparation shows less matrix interference with the analysis compared to honey samples. An example of an analysis of shrimp is shown in Figure 7. Due to the reduced matrix effect, the LOD with the quadrupole is lowered to nearly the same level as for the trap (0.05 ppb in the sample with the five-fold concentration). A concentration of 0.35 ppb was recovered in the shrimp sample by both the quadrupole and the trap MSD. Extraction recovery was approximately 85%.

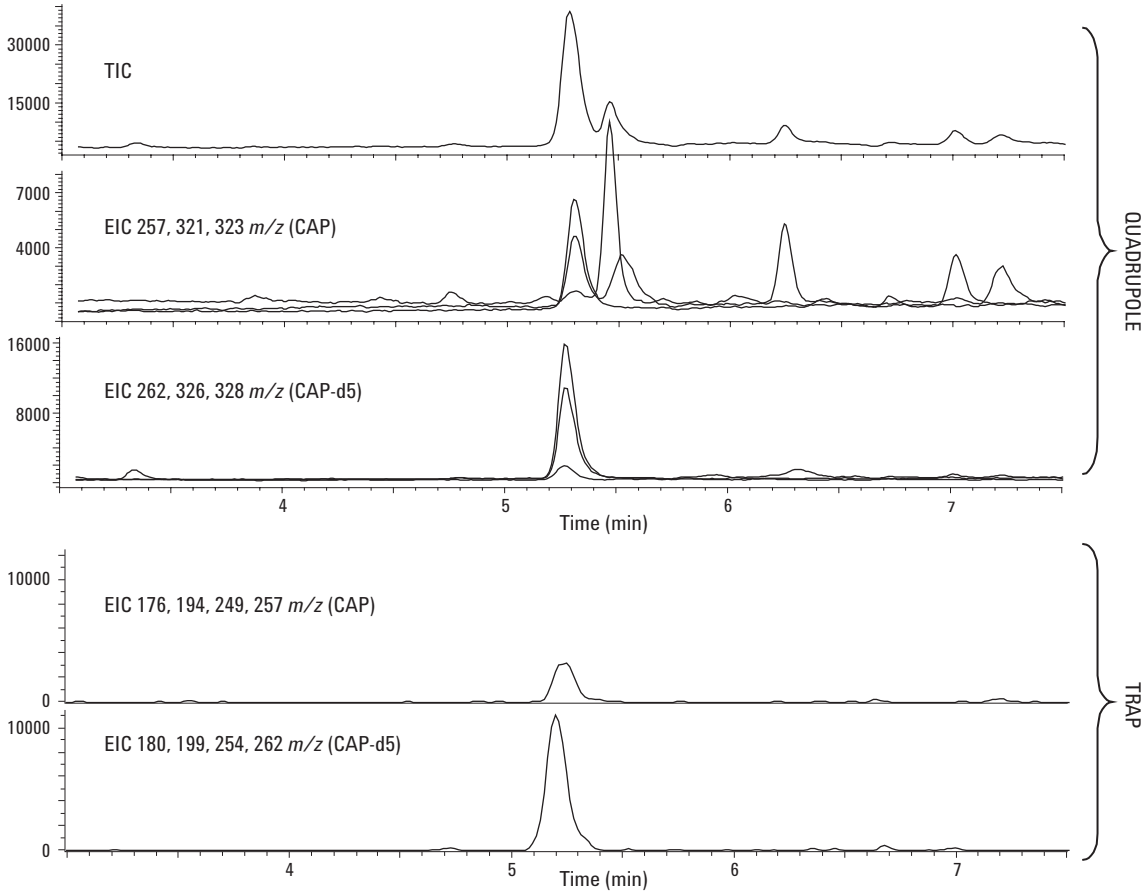


Figure 7. Analysis of a shrimp containing 0.35 ppb CAP and 1 ppb CAP-d5.

Conclusion

Honey and shrimp samples were successfully analyzed for CAP with both the quadrupole and trap MSD. A simple liquid-liquid extraction procedure using ISOLUTE HM-N cartridges was found to perform excellently in view of recovery and repeatability. The LC method used a standard 4.6-mm id column and produced the required sensitivity on both instruments. The LC/MSD quadrupole instrument produced excellent linearity and demonstrated its quantitative ability. The LC/MSD Trap showed the needed sensitivity with excellent full scan capability below the regulated limit in both sample matrices. The use of a broad isolation window for full scan spectra using the ion trap produced more transition ions than required for confirmation.

References

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